INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Martina *et al.*, *Int J Syst Evol Microbiol* 2018;68:14–20
DOI 10.1099/ijsem.0.002293

MICROBIOLOGY SOCIETY

Burkholderia puraquae sp. nov., a novel species of the *Burkholderia cepacia* complex isolated from hospital settings and agricultural soils

Pablo Martina,[1]†‡ Mariana Leguizamon,[1]† Claudia I. Prieto,[1] Silvia A. Sousa,[2] Patricia Montanaro,[3] Walter O. Draghi,[4,5] Maren Stämmler,[6] Marisa Bettiol,[7] Carla C. C. R. de Carvalho,[2] Juliana Palau,[7] Cecilia Figoli,[1] Florencia Alvarez,[5] Silvina Benetti,[8] Sergio Lejona,[8] Cecilia Vescina,[7] Julián Ferreras,[9] Peter Lasch,[6] Antonio Lagares,[5] Angeles Zorreguieta,[4] Jorge H. Leitão,[2] Osvaldo M. Yantorno[1] and Alejandra Bosch[1,*]

Abstract

Bacteria from the *Burkholderia cepacia* complex (Bcc) are capable of causing severe infections in patients with cystic fibrosis (CF). These opportunistic pathogens are also widely distributed in natural and man-made environments. After a 12-year epidemiological surveillance involving Bcc bacteria from respiratory secretions of Argentinean patients with CF and from hospital settings, we found six isolates of the Bcc with a concatenated species-specific allele sequence that differed by more than 3 % from those of the Bcc with validly published names. According to the multilocus sequence analysis (MLSA), these isolates clustered with the agricultural soil strain, *Burkholderia* sp. PBP 78, which was already deposited in the PubMLST database. The isolates were examined using a polyphasic approach, which included 16S rRNA, *recA*, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), DNA base composition, average nucleotide identities (ANIs), fatty acid profiles, and biochemical characterizations. The results of the present study demonstrate that the seven isolates represent a single novel species within the Bcc, for which the name *Burkholderia puraquae* sp. nov. is proposed. *Burkholderia puraquae* sp. nov. CAMPA 1040^T (=LMG 29660^T=DSM 103137^T) was designated the type strain of the novel species, which can be differentiated from other species of the Bcc mainly from *recA* gene sequence analysis, MLSA, ANIb, MALDI-TOF MS analysis, and some biochemical tests, including the ability to grow at 42 °C, aesculin hydrolysis, and lysine decarboxylase and β-galactosidase activities.

The *Burkholderia cepacia complex* (Bcc) is a closely related group of bacteria, which are ubiquitous in nature [1–3]. At the time of writing this report, a total of 20 different species with validly published names have been assigned to the Bcc [2–4]. The versatile lifestyle exhibited by species of the Bcc enables them to colonize and infect a wide variety of habitats including rhizospheric soil, plant root nodules, freshwater sediments, industrial products, dialysis water, medical instruments and hospital settings [5, 6]. Species of the Bcc are highly problematic human pathogens causing severe

Author affiliations: [1]CINDEFI, CONICET-CCT La Plata, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina; [2]iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; [3]Hospital Santísima Trinidad de Córdoba, Córdoba, Argentina; [4]Fundación Instituto Leloir and IIBA-Consejo Nacional de Investigaciones Científicas y Tecnológicas, Buenos aires, Argentina; [5]IBBM, CONICET-CCT La Plata, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina; [6]Proteomics and Spectroscopy Unit (ZBS6) at the Centre for Biological Threats and Special Pathogens, Robert Koch-Institut, Berlin, Germany; [7]Sala de Microbiología, Hospital de Niños «Sor María Ludovica», La Plata, Buenos Aires, Argentina; [8]Laboratorio Cemar D. S. L. A. C, Municipalidad de Rosario, Santa Fe, Argentina; [9]IBS, CONICET-CCT Nordeste, Facultad de Ciencias Exactas Químicas y Naturales, Universidad Nacional de Misiones, Posadas, Misiones, Argentina.
***Correspondence:** Alejandra Bosch, bosch@quimica.unlp.edu.ar
Keywords: *Burkholderia* genus; *Burkholderia cepacia* complex; *Burkholderia puraquae* sp. nov.
Abbreviations: ANIb, average nucleotide identity based on BLAST; Bcc, *Burkholderia cepacia* complex; CF, cystic fibrosis; FAME, Fatty acid methyl esters; MALDI-TOF MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MLSA, multi-locus sequence analysis; ST, sequence type; UHCA, Unsupervised hierarchical cluster analysis; WGS, Whole genome sequencing.
†These authors contributed equally to this work.
‡Present address: IBS, CONICET-CCT Nordeste, Facultad de Ciencias Exactas Químicas y Naturales, Universidad Nacional de Misiones, Posadas, Misiones, Argentina.
The GenBank accession numbers for the 16S rRNA, *hisA* and *recA* gene sequences of *Burkholderia puraquae* sp. nov. CAMPA 1043 are: KX516808, KX516814, KX516844, respectively. Those for the 16S rRNA, hisA and recA gene sequences of *Burkholderia puraquae* sp. nov. CAMPA 1040^T (=LMG 29660^T, DSM 103137^T) are: KX278717, KX516813, KX516843, respectively. The GenBank accession numbers for the 16S rRNA, *hisA* and *recA* gene

respiratory infections in patients with cystic fibrosis (CF), although other hospitalized non-CF patients have also been affected [5, 7] and a number of outbreaks have been reported in recent decades [6, 8–10].

It is known that the 16S rRNA gene, which is widely used for bacterial systematics, is limited in its ability to differentiate between members of the Bcc [11]. In contrast, the analysis of *recA* and *hisA* sequence variations has enabled the identification of most of the species within the complex [12, 13]. In comparison with these methods, however, the multilocus sequence typing (MLST) scheme provides both species and strain identification [14]. Accordingly, multilocus sequence analysis (MLSA) has been used to elucidate intraspecies relationships between members of the *Burkholderia* genus, and to analyze strains within species [3, 15]. In this respect it was demonstrated that a 3 % divergence among concatenated allele sequences can be used as a threshold value for species delineation within the Bcc [2]. However, the introduction of whole genome sequencing (WGS) allows the implemention of complementary taxonomic tools to MLSA, such as the average nucleotide identity (ANI) [16, 17]. This WGS-based approach has become one of the most robust measurements of genomic relatedness for discriminating both distant and closely related bacteria.

During an epidemiological surveillance involving Bcc bacteria from respiratory secretions of CF patients, and from hospital settings representing different CF reference centres in Argentina [9, 18], we recovered six isolates from hospital settings that represented a unique MLSA cluster within the Bcc. Furthermore, after depositing the sequences in the Bcc PubMLST database (http://pubmlst.org/bcc/), one additional Bcc isolate was identified as belonging to the same group, the PBP 78 isolate, recovered from an agricultural soil sample in Argentina in 2011. The aim of the present study was, therefore, to analyze the taxonomic position of these Bcc-like isolates using a polyphasic approach.

The six isolates from hospital settings were deposited in the CAMPA Collection (Colección Argentina de Microorganismos Patógenos y Ambientales) at CINDEFI, CONICET-CCT La Plata (Table 1). Five of these (isolates CAMPA 565, CAMPA 567, CAMPA 707, CAMPA 1040^T, and CAMPA 1043) were recovered in the period 2007 to 2012 from haemodialysis water reservoirs at the Hospital Santísima Trinidad in Córdoba city (31° 25′ 00″ S 64° 11′ 00″ O), Argentina. The remaining isolate, CAMPA 566, was isolated in 2009 from a haemodialysis water reservoir at a hospital in San Fernando del Valle de Catamarca (28° 28′ 07″ S 65° 46′ 45″ O) in Catamarca Province, 438 km from Córdoba. In both hospitals, the occurrence of bacterial contamination in tubes, tanks and taps of haemodialysis units is investigated monthly. Membrane filtration (pore size of membranes, 0.22 µm), was used as the concentrating technique, according to the water examination standard of the American Public Health Association (APHA) [19]. Following filtration, membranes were placed in enrichment medium and incubated at 37 °C for 24 h. Positive cultures were inoculated on *Burkholderia cepacia* selective agar plates (BCSA, Britannia) and incubated for 3 days at 37 °C and for 2 additional days at room temperature. Routine biochemical and molecular characterization of colonies of isolates were carried out. Isolate PBP 78 was obtained from a soil sample recovered in Pergamino, Buenos Aires province, (33°53′ 00″ S 60°34′ 00″ O), 512 km from Córdoba city, following environmental sampling and bacterial isolation procedures described previously [20] (Table 1).

Stock cultures were prepared with bacteria grown for 24 h on Tryptone Soya Agar (Oxoid), incubated at 36 °C. Cultures were preserved as both lyophilized and frozen stocks at −80 °C in LB medium with 20 % (v/v) glycerol until further analysed. Genomic DNA was prepared for PCR experiments as previously described [9].

The nearly complete sequences of the 16S rRNA gene of the isolates were amplified by PCR using the conserved primers 27 f and 1492 r [21]. The DNA sequencing of the 16S rRNA gene was performed at Macrogen (Seoul, South Korea) using the BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems). Sequence assembly was carried out by using Vector NTI advance 10.1 software. Sequences of all isolates and related type strains were aligned using CLUSTAL W software. Phylogenetic analysis was conducted in MEGA 6 [22]. Pairwise comparison of these sequences against those from reference strains of other species of the Bcc revealed similarity levels of between 98.8 and 99.7 %. Similarity values against *Burkholderia gladioli* and *Burkholderia glumae* were in the range of 98.4–98.8 %, while similarities with other species of the genus *Burkholderia* were below 95.2 % (data not shown). The phylogenetic tree inferred from the 16S rRNA sequences showed that the seven isolates grouped within the Bcc in a highly (86 %) supported cluster. (Fig. S1, available in the online version of this article).

The *recA* gene (1041 bp) was amplified with BCR1 and BCR2 primers [11]. Sequence analysis and assembly were performed for the seven isolates as described above for the 16S rRNA gene. The *recA* sequences obtained showed similarity values of between 89.5 and 94.7 % between the seven

sequences of *Burkholderia puraquae* sp. nov. CAMPA 707 are: KX516807, KX516812, KX516842, respectively. The GenBank accession numbers for the 16S rRNA, *hisA* and *recA* gene sequences of *Burkholderia puraquae* sp. nov. CAMPA 567 are: KX516806, KX516811, KX516841, respectively. Those for the 16S rRNA, *hisA* and *recA* gene sequences of *Burkholderia puraquae* sp. nov. CAMPA 566 are: KX516805, KX516810, KX516840 respectively. The GenBank accession numbers for the 16S rRNA, *hisA* and *recA* gene sequences of *Burkholderia puraquae* sp. nov. CAMPA 565 are: KX516804, KX516809, KX516839, respectively. The GenBank accession numbers for the 16S rRNA and *recA* gene sequences of *Burkholderia puraquae* sp. nov. PBP78 are: KX534057, KX534063, respectively. The draft genome sequences of *Burkholderia* sp. CAMPA 1040^T was deposited in DDBJ/EMBL/GenBank under the accession numbers NBYX00000000.

One supplementary table and four supplementary figures are available with the online version of this article.

Table 1. Isolates studied showing sources, sequence types and allelic profiles

Nucleotide sequences of each allele, allelic profiles and sequence types for all isolates are available on the Bcc PubMLST website (http://pubmlst.org/bcc). CAMPA: Colección Argentina de Microorganismos Patógenos y Ambientales, CINDEFI, CONICET-CCT La Plata, National University of La Plata, Argentina; HST 245, name given by 'Hospital Santísima Trinidad de Córdoba' (HST) to the CAMPA 1040^T isolate; Isolate PBP 78, obtained from Molecular and Cellular Microbiology Laboratory, Fundación Instituto Leloir, Argentina; ST, sequence type.

Isolates*,†,‡	Source, isolation site, year	ST	Allelic profile *Burkholderia cepacia* complex MLST						
			atpD	*gltB*	*gyrB*	*recA*	*lepA*	*phaC*	*trpB*
CAMPA 565	Dialysis water, Córdoba, 2009	1065	378	451	690	404	459	290	450
CAMPA 566	Dialysis water, Catamarca, 2009	1065	378	451	690	404	459	290	450
CAMPA 567	Dialysis water, Córdoba, 2009	1065	378	451	690	404	459	290	450
CAMPA 707	Dialysis water, Córdoba, 2007	1065	378	451	690	404	459	290	450
HST 245 CAMPA 1040^T LMG 29660^T DMSZ 103137 ^{T}	Dialysis water, Córdoba, 2011	1065	378	451	690	404	459	290	450
CAMPA 1043	Dialysis water, Córdoba, 2012	1065	378	451	690	404	459	290	450
PBP 78 R-50214	Soil, Buenos Aires, 2011	764	316	363	548	334	381	290	66

candidates and species of the Bcc with validly published names. The phylogenetic tree derived showed a tight clustering (99 %) of the seven candidate strains within the Bcc, as previously observed in the 16S rRNA gene tree (Fig. S2).

As phylogenetic analysis of the 442 bp *hisA* gene fragment is an additional powerful tool for discriminating between species of the Bcc [13], PCR amplification of the *hisA* region and its nucleotide sequence was determined. Pairwise comparison of these sequences with those of the other members of the Bcc revealed similarity levels between 91.08 and 97.14 % (data not shown). A phylogenetically derived tree showed that the six isolates recovered from the hospital settings were grouped in a cluster within the Bcc (Fig. S3). Furthermore, we determined the 11 letter code obtained from the combination of the nucleotides within the *hisA* gene, which has been reported to allow discrimination between species of the Bcc [13]. The 6 isolates presented the same 11 letter code for this site –CACGGCGGCTA–, which was different from other species of Bcc with validly published names.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used to discriminate and identify members of the Bcc from other non-fermentative rods normally recovered from the sputum cultures of CF patients [23, 24] as well as within the Bcc down to the species level [15, 25–27]. The seven isolates studied here, 17 strains from different species of the genus *Burkholderia*, and six non-fermentative rods not belonging to the genus *Burkholderia* (see Fig. S4 for details), were grown on TSA for 24 h at 37 °C, and mass spectra were acquired with an Autoflex I mass spectrometer (Bruker Daltonics) using the FlexControl v.3.0 software for data acquisition (Bruker) [24]. A spectral database was constructed with at least three biological/technical MALDI-TOF mass replicate spectra of the individual strains or isolates mentioned above. When an identification analysis of the isolates was carried out by means of the commercial system Bruker Daltonik MALDI Biotyper (Bruker), *Burkholderia pyrrocinia* was the identification obtained for the seven isolates with score values ranging from 2.41 to 2.38, depending on the isolate. *Burkholderia cenocepacia* and *Burkholderia stabilis* were the candidates that followed in the identification list. Each mass spectral pattern of the isolates and reference strains indicated above, were further also analyzed by the Matlab-based (The Mathworks) software package, MicrobeMS [28]. By means of MicrobeMS, an unsupervised hierarchical cluster analysis (UHCA) was performed using the MALDI-TOF mass spectral database obtained with the reference strains indicated above, and the seven isolates. For UHCA, the spectral information in the m/z region of 2000–14 000 was extracted and converted to barcode spectra, according to the procedures described by Lasch and Naumann [28, 29], and Ward's algorithm was used as the clustering method. Fig. S4 shows the dendrogram of the cluster analysis carried out with a total of 62 database spectra from the 18 different species of the genus *Burkholderia* (including database spectra from the seven isolates) and MALDI-TOF mass spectra from six non-fermentative rods not belonging to the genus *Burkholderia*. These results show that these seven isolates were grouped in a distinct cluster included in an 'a2' cluster, within the Bcc reference stains and *Burkholderia gladioli* (cluster A), indicating that they can be distinguished from the most relevant species of the Bcc, from other species of the genus *Burkholderia*, and from other non-fermentative rods (cluster B) (Fig. S4).

The *recA* and *hisA* gene sequence similarity levels towards other reference strains of species of the Bcc and the comparison of the MALDI-TOF mass spectra of the seven isolates against the more relevant species of the Bcc, show that these isolates comprise a tightly cluster of distinctive strains within the Bcc taxon. Thus, MLSA was performed using

standard protocols [14] to confirm whether they represent a novel species within the complex. A phylogenetic tree, based on the concatenated sequences (2773 bp) of seven housekeeping gene fragments [*atpD* (443 bp), *gltB* (400 bp), *gyrB* (454 bp), *recA* (393 bp), *lepA* (397 bp), *phaC* (385 bp) and *trpB* (301 bp)], was reconstructed using MEGA 6. The mean number of nucleotide substitutions per site (i.e. the percentage of divergence of concatenated allele sequences) between Bcc with validly published names and the seven isolates was calculated as previously described [2]. The isolates were resolved into two sequence types (STs), with CAMPA 1040^T and PBP 78 as their corresponding representatives (Table 1). The nucleotide sequence of each allele, allelic profiles and STs of these two strains are available on the Bcc PubMLST database. The phylogenetic analysis of concatenated allele sequences demonstrated that the seven isolates grouped together in one cluster within the Bcc, supported by bootstrap values of 100 % (Fig. 1). For the novel taxon, the concatenated-allele intra-group sequence divergence was 1.75 %, while the mean divergence with the closest neighbours was 4.17±1.71 % to *Burkholderia metallica*, 4.79 ±1.18 % to *Burkholderia contaminans* and 4.81±1.29 % to *Burkholderia lata*.

ANI values have become a gold standard in modern bacterial taxonomy for the determination of species delineation [16, 17]. Based on WGS data, ANIb was performed to estimate the mean values between homologous genomic regions shared by CAMPA 1040^T and the nearest species of the Bcc, according to the MLSA phylogenetic tree (Fig. 1). This results demonstrated that *B. puraquae* sp. *nov.* CAMPA 1040^T showed ANIb-values of 92.68 and 92.84 % against *B. contaminans LMG 23361* and *B. lata LMG 22485,* respectively. As ANI values of 95 % are considered to be the boundary for species delineation [17, 30], these results and the MLSA derived analysis confirm that the seven isolates represent a novel species of the Bcc.

The DNA G+C content (mol%) determination was performed at DSMZ (Germany). The values obtained for CAMPA 565, CAMPA 566, CAMPA 567, CAMPA 707, CAMPA 1040^T and CAMPA 1043 isolates were 66.2, 66.1, 66.4, 66.8, 66.3 and 66.7 mol%, respectively, which are within the range reported for other species of the Bcc (66–69 mol%) [31].

Fatty acid analysis was performed using the Sherlock Microbial Identification System (MIS) (MIDI, Newark, USA), after growing each isolate on TSA plates at 30 °C for 24 h, as described previously [32]. Briefly, after harvesting the cells, fatty acids were extracted and methylated to fatty acid methyl esters (FAME) by using the Instant FAME procedure from MIDI. FAME composition was determined by gas chromatography using Sherlock software version 6.2. The most abundant fatty acids, both in the isolates and in the reference strains that are neighbours in the phylogenetic MLSA tree, *B. contaminans* LMG 23361 and *B. lata* LMG 22485, were 18 : 1ω7*c* and/or 19 : 0 cycloω8*c* (in Table S1 as summed feature 8), 16 : 1ω7*c* and/or 16 : 1ω6*c* (in Table S1 as summed feature 3) and 16 : 0, corresponding to over 89 % of the total content of fatty acids (Table S1). Analysis of the same samples using the phospholipid fatty acid analysis method of Sherlock on a gas chromatograph with a mass spectrometer detector, as described previously [32] showed that summed feature 8 contained both 18 : 1ω7*c* and 19 : 0 cycloω8*c*, but summed features 2, 3 and 5 contained only 14 : 0 3OH, 16 : 1ω7*c* and 18 : 2ω6, 9*c*, respectively (data not shown). Both principal component analysis (PCA) carried out with Sherlock software to assess strain proximity, and calculations carried out according to Vauterin *et al.* [33] to determine fatty acid profile homogeneity, indicated that the lipid composition of all isolates reported here are different to reference strains that are phylogenetically neighbouring species in the MLSA tree, whilst displaying a certain degree of heterogeneity amongst themselves (the sum of the differences between each fatty acid and the average of isolates varied up to 3.2 %).

The biochemical characterization for the seven isolates, performed as described by Henry *et al.* [34] is indicated in Table 2. The strains assimilate glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-gluconate, caprate, adipate, L-malate, citrate and phenylacetate, but do not assimilate maltose. Oxidase, β-galactosidase, aesculin hydrolase and lysine decarboxylase activities are present, but not ornithine decarboxylase, arginine dihydrolase and urease. Nitrate reduction and gelatin liquefaction were strain-dependent. The seven isolates studied could be differentiated from other species of Bcc mainly because they do not grow at 42 °C as almost all the other species of Bcc do. The isolates described here were able to grow on BCSA medium, assimilated xylose, they were negative for arginine dihydrolase activity and did not show β-haemolysis on blood agar plates. VITEK identification [35] yielded 'very good' to 'excellent' identification (93–98 %) as *Burkholderia cepacia* for the whole set of isolates.

In summary, *recA* gene sequence analysis, MALDI-TOF mass spectrometry, MLSA, whole-genome ANIb, and some biochemical tests, including the ability to grow at 42 °C, hydrolysis of aesculin, and lysine decarboxylase and β-galactosidase activity, confirmed that CAMPA 565, CAMPA 566, CAMPA 567, CAMPA 707, CAMPA 1040^T, CAMPA 1043 and PBP 78 are a distinguishable, novel, group of bacteria within the members of the Bcc. It is, therefore, proposed to classify these isolates with the name *Burkholderia puraquae* sp. nov., with strain CAMPA 1040^T (=LMG 29660^T=DSM 103137 ^{T}) as the type strain.

DESCRIPTION OF *BURKHOLDERIA PURAQUAE* SP. NOV.

Burkholderia puraquae (pur.a.quae. L. adj. *purus -a -um pure*; L. n. *aqua* water; N.L. gen. n. *puraquae* of pure water).

Cells are Gram-stain-negative, aerobic, non-sporulating rods. Generally colonies are moist and shiny. All characterized isolates grow in the range 30 to 37 °C on MacConkey

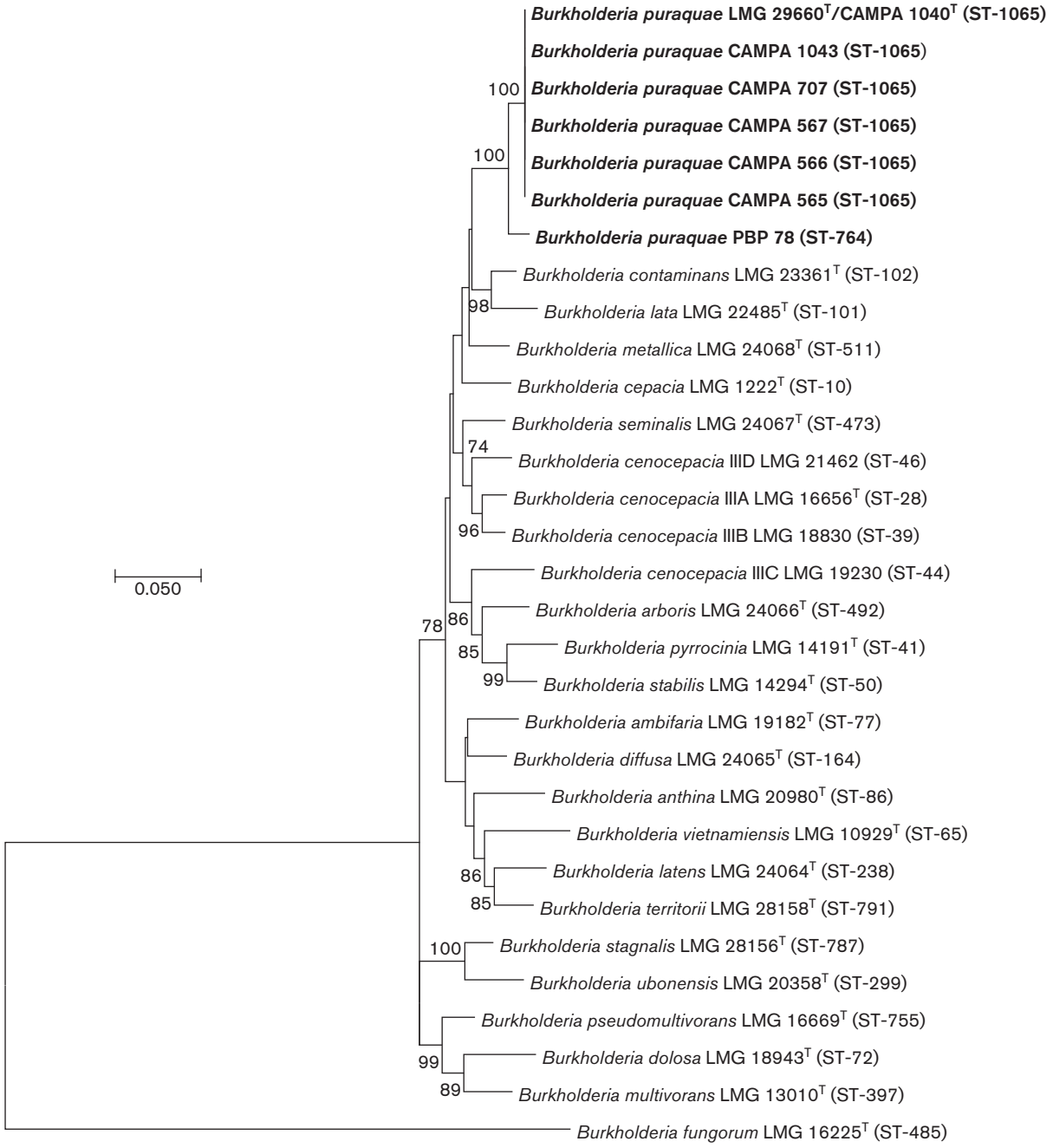

Fig. 1. Phylogenetic tree, based on the concatenated sequences (2773 bp) of seven housekeeping gene fragments of species of the Bcc with validly published names and isolates of *Burkholderia puraquae* sp. nov. The bootstrap consensus tree, inferred from 1000 replicates, was reconstructed using the maximum-likelihood method based on the General Time Reversible model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches if greater than 70 %. A discrete Gamma distribution was used to model evolutionary rate differences among sites 5 categories (+G, parameter=0.1884) and allowed for some sites to be evolutionarily invariable ([+I], 52.2421 % sites). The sequence of *Burkholderia fungorum* LMG 16225T was used as the outgroup. Scale bar, number of substitutions per site.

agar and on BCSA, while growth at 42 °C is not observed. All isolates are yellow, and they are not haemolytic. The isolates assimilate glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-gluconate, caprate, adipate, L-malate, citrate and phenylacetate, but do not assimilate maltose. Acidification of glucose, mannitol, lactose and xylose is observed. Nitrate reduction is not present. Oxidase, β-galactosidase, aesculin hydrolase, lysine decarboxylase

Table 2. Biochemical characteristics differentiating *B. puraquae* sp. nov. from other members of the *Burkholderia cepacia* complex

Species: 1, *Burkholderia puraquae* sp. nov. CAMPA 565, CAMPA 566, CAMPA 567, CAMPA 707, CAMPA 1040^T, CAMPA 1043 and PBP 78; 2, *Burkholderia contaminans*; 3, *Burkholderia lata*; 4, *Burkholderia metallica*; 5, *Burkholderia cepacia*; 6, *Burkholderia seminalis*; 7, *Burkholderia cenocepacia*; 8, *Burkholderia multivorans*; 9, *Burkholderia ambifaria*; 10, *Burkholderia diffusa*; 11, *Burkholderia pyrrocinia*; 12, *Burkholderia pseudomultivorans*; 13, *Burkholderia latens*; 14, *Burkholderia arboris*; 15, *Burkholderia stabilis*; 16, *Burkholderia vietnamiensis*; 17, *Burkholderia dolosa*; 18, *Burkholderia anthina*; 19, *Burkholderia ubonensis*; 20, *Burkholderia stagnalis*; 21, *Burkholderia territorii*. +, >90% of all isolates positive; v, 10–90 % positive; −,<10 % of strains positive; w, weak reaction; y, yellow. Phenotypic characteristics for *Burkholderia puraquae* sp. nov. were obtained in the present study and results for the other strains were taken from De Smet *et al.* [4].

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Growth on Mc Conkey agar	+	+	+	+	v	+	v	+	+	+	+	+	+	+	+	v	+	+	+	+	+
Growth at 42°	−	v	−	+	v	+	v	+	v	v	v	+	+	v	−	+	+	v	v	+	+
Pigment	y	v	v	y	v	v	v	−	v	−	v	−	−	v	−	−	−	−	−	−	−
Haemolysis	−	v	−	−	−	−	−	−	v	−	v	−	−	v	−	v	−	−	−	−	−
OF Manitol	+	+	+	+	+	+	+	+	+	+	v	+	+	+	+	+	+	+	+	+	+
OF Lactose	+	+	+	+	+	+	v	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OF Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	w	v	v	+	+	+	+	+
Nitrate reduction	−	v	v	−	−	−	v	+	v	+	v	v	−	v	−	v	+	v	v	−	−
Lysine decarboxylase	+	+	+	+	+	v	+	v	+	+	+	+	+	v	+	+	−	v	−	+	+
Ornithine decarboxylase	−	−	v	−	v	v	v	−	−	−	+	−	−	+	+	−	−	v	−	−	−
Arginine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Gelatinase	4(+)	+	v	+	v	+	v	−	+	v	+	−	v	+	v	−	−	−	+	+	+
β-Galactosidase	+	+	v	+	+	+	+	+	+	+	+	+	+	+	−	+	+	v	−	−	+
Aesculin hydrolysis	+	v	v	+	v	v	v	−	v	−	−	v	−	−	−	−	−	−	−	−	−

activities are present, but not ornithine decarboxylase, arginine dihydrolase and urease. Gelatin liquefaction is isolate-dependent (four of seven). The following fatty acids are present in all isolates: 12 : 0, 14 : 0, 14 : 0 3OH, 16 : 0, 16 : 1 2OH, 16 : 1ω5*c*, 16 : 1ω7c, 17 : 0, 18 : 0, 18 : 1 2OH, 18 : 1ω5*c*, 18 : 1ω7*c*, 18 : 2ω6,9*c*, 18 : 3ω6*c* (6,9,12), and 19 : 0 cycloω8*c*.

The type strain (LMG 29660^T=DSM 103137^T), originally collected as CAMPA 1040^T, was isolated from haemodialysis water in Córdoba province, Argentina, and it is gelatin liquefaction positive, with all remaining phenotypic properties similar to other isolates of the species. The DNA G+C content of the type strain is 66.3 mol%.

Funding information
CCCR de Carvalho acknowledges Fundação para a Ciência e a Tecnologia (Portugal) for financial support under program 'FCT Investigator 2013' (IF/01203/2013/CP1163/CT0002). SAS acknowledges a Post-doctoral fellowship from FCT (SFRH/BPD/102006/2014). Funding received by iBB from FCT (UID/BIO/04565/2013) and Programa Operacional Regional de Lisboa 2020 (Project N. 007317) is acknowledged. Bosch A. acknowledges Universidad Nacional de La Plata for financial support, Project: 11/X657. A. Bosch is member of Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC-PBA).

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Ethical approval to report this manuscript was not required.

References

1. Coenye T, Vandamme P. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environ Microbiol* 2003;5:719–729.
2. Vanlaere E, Baldwin A, Gevers D, Henry D, De Brandt E *et al.* Taxon K, a complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. *Int J Syst Evol Microbiol* 2009; 59:102–111.
3. Peeters C, Zlosnik JE, Spilker T, Hird TJ, LiPuma JJ *et al.* *Burkholderia pseudomultivorans* sp. nov., a novel *Burkholderia cepacia* complex species from human respiratory samples and the rhizosphere. *Syst Appl Microbiol* 2013;36:483–489.
4. De Smet B, Mayo M, Peeters C, Zlosnik JE, Spilker T *et al.* *Burkholderia stagnalis* sp. nov. and *Burkholderia territorii* sp. nov., two novel *Burkholderia cepacia* complex species from environmental and human sources. *Int J Syst Evol Microbiol* 2015;65:2265–2271.
5. Baldwin A, Mahenthiralingam E, Drevinek P, Vandamme P, Govan JR *et al.* Environmental *Burkholderia cepacia* complex isolates in human infections. *Emerg Infect Dis* 2007;13:458–461.
6. Martin M, Christiansen B, Caspari G, Hogardt M, von Thomsen AJ *et al.* Hospital-wide outbreak of *Burkholderia contaminans* caused by prefabricated moist washcloths. *J Hosp Infect* 2011;77:267–270.
7. Drevinek P, Mahenthiralingam E. *Burkholderia cenocepacia* in cystic fibrosis: epidemiology and molecular mechanisms of virulence. *Clin Microbiol Infect* 2010;16:821–830.
8. Abe K, D'Angelo MT, Sunenshine R, Noble-Wang J, Cope J *et al.* Outbreak of *Burkholderia cepacia* bloodstream infection at an outpatient hematology and oncology practice. *Infect Control Hosp Epidemiol* 2007;28:1311–1313.
9. Martina P, Bettiol M, Vescina C, Montanaro P, Mannino MC *et al.* Genetic diversity of *Burkholderia contaminans* isolates from cystic fibrosis patients in Argentina. *J Clin Microbiol* 2013;51:339–344.
10. Heo ST, Kim SJ, Jeong YG, Bae IG, Jin JS *et al.* Hospital outbreak of *Burkholderia stabilis* bacteraemia related to contaminated chlorhexidine in haematological malignancy patients with indwelling catheters. *J Hosp Infect* 2008;70:241–245.
11. Mahenthiralingam E, Bischof J, Byrne SK, Radomski C, Davies JE *et al.* DNA-Based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*,

Burkholderia multivorans, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. *J Clin Microbiol* 2000;38:3165–3173.

12. **Mahenthiralingam E, Baldwin A, Vandamme P.** *Burkholderia cepacia* complex infection in patients with cystic fibrosis. *J Med Microbiol* 2002;51:533–538.
13. **Papaleo MC, Perrin E, Maida I, Fondi M, Fani R *et al.*** Identification of species of the *Burkholderia cepacia* complex by sequence analysis of the *hisA* gene. *J Med Microbiol* 2010;59:1163–1170.
14. **Baldwin A, Mahenthiralingam E, Thickett KM, Honeybourne D, Maiden MC *et al.*** Multilocus sequence typing scheme that provides both species and strain differentiation for the *Burkholderia cepacia* complex. *J Clin Microbiol* 2005;43:4665–4673.
15. **Vanlaere E, LiPuma JJ, Baldwin A, Henry D, De Brandt E *et al.*** *Burkholderia latens* sp. nov., *Burkholderia diffusa* sp. nov., *Burkholderia arboris* sp. nov., *Burkholderia seminalis* sp. nov. and *Burkholderia metallica* sp. nov., novel species within the *Burkholderia cepacia* complex. *Int J Syst Evol Microbiol* 2008;58:1580–1590.
16. **Konstantinidis KT, Tiedje JM.** Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci USA* 2005; 102:2567–2572.
17. **Richter M, Rosselló-Móra R.** Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
18. **Martina P, Feliziani S, Juan C, Bettiol M, Gatti B *et al.*** Hypermutation in *Burkholderia cepacia* complex is mediated by DNA mismatch repair inactivation and is highly prevalent in cystic fibrosis chronic respiratory infection. *Int J Med Microbiol* 2014;304:1182–1191.
19. **American Public Health Association.** *Standard Methods for the Examination of Water and Wastewater*, 21st ed. Washington, DC: APHA; 2005.
20. **Draghi WO, Peeters C, Cnockaert M, Snauwaert C, Wall LG *et al.*** *Burkholderia cordobensis* sp. nov., from agricultural soils. *Int J Syst Evol Microbiol* 2014;64:2003–2008.
21. **Lane DJ.** 16S/23S rRNA sequencing. In: Stackebrandt EGM (editor). *Nucleic Acid Techniques in Bacterial systematics*. New York: John Wiley & Sons, Inc.; 1991. pp. 115–176.
22. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
23. **Desai AP, Stanley T, Atuan M, Mckey J, LiPuma JJ *et al.*** Use of matrix assisted laser desorption ionisation-time of flight mass spectrometry in a paediatric clinical laboratory for identification of bacteria commonly isolated from cystic fibrosis patients. *J Clin Pathol* 2012;65:835–838.
24. **Miñán A, Bosch A, Lasch P, Stämmler M, Serra DO *et al.*** Rapid identification of *Burkholderia cepacia* complex species including strains of the novel Taxon K, recovered from cystic fibrosis patients by intact cell MALDI-ToF mass spectrometry. *Analyst* 2009;134:1138–1148.
25. **Lambiase A, del Pezzo M, Cerbone D, Raia V, Rossano F *et al.*** Rapid identification of *Burkholderia cepacia* complex species recovered from cystic fibrosis patients using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *J Microbiol Methods* 2013;92:145–149.
26. **Lasch P, Naumann D.** MALDI-TOF mass spectrometry for the rapid identification of highly pathogenic microorganisms. In: Jiri Stulik J, Toman R, Butaye P and Ulrich R (editors). *Proteomics, Glycomics and Antigenicity of BSL3 and BSL4 Agents*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; 2011. pp. 212–219.
27. **Mellmann A, Cloud J, Maier T, Keckevoet U, Ramminger I *et al.*** Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification of nonfermenting bacteria. *J Clin Microbiol* 2008;46:1946–1954.
28. **Lasch P, Naumann D.** Infrared spectroscopy in microbiology. *Encyclopedia of Analytical Chemistry*. Berlin, Germany: John Wiley & Sons, Ltd; 2015. pp. 1–32.
29. **Lasch P.** MicrobeMS: A Matlab toolbox for analysis of microbial MALDI-TOF mass spectra. 2017. www.microbe-ms.com.
30. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P *et al.*** DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
31. **Vandamme P, Dawyndt P.** Classification and identification of the *Burkholderia cepacia* complex: past, present and future. *Syst Appl Microbiol* 2011;34:87–95.
32. **De Carvalho CC, Caramujo MJ.** Bacterial diversity assessed by cultivation-based techniques shows predominance of *Staphylococccus* species on coins collected in Lisbon and Casablanca. *FEMS Microbiol Ecol* 2014;88:26–37.
33. **Vauterin L, Yang P, Swings J.** Utilization of fatty acid methyl esters for the differentiation of new *Xanthomonas* species. *Int J Syst Bacteriol* 1996;46:298–304.
34. **Henry DA, Mahenthiralingam E, Vandamme P, Coenye T, Speert DP.** Phenotypic methods for determining genomovar status of the *Burkholderia cepacia* complex. *J Clin Microbiol* 2001; 39:1073–1078.
35. **Ligozzi M, Bernini C, Bonora MG, de Fatima M, Zuliani J *et al.*** Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. *J Clin Microbiol* 2002;40:1681–1686.